Resource Handbook $.50

DIGITAL EQUIPMENT CORPORATION

education

HUNTINGTON II Simulation Program — LOCKEY

computers are for kids — digital — EduSystems—expandable, economical

DIGITAL EQUIPMENT CORPORATION
EDUCATIONAL PUBLICATIONS

A partial list of the publications in the continuing series of curriculum material published by Digital for use with EduSystems and RSTS are listed below. Please inquire directly for prices on classroom quantities.

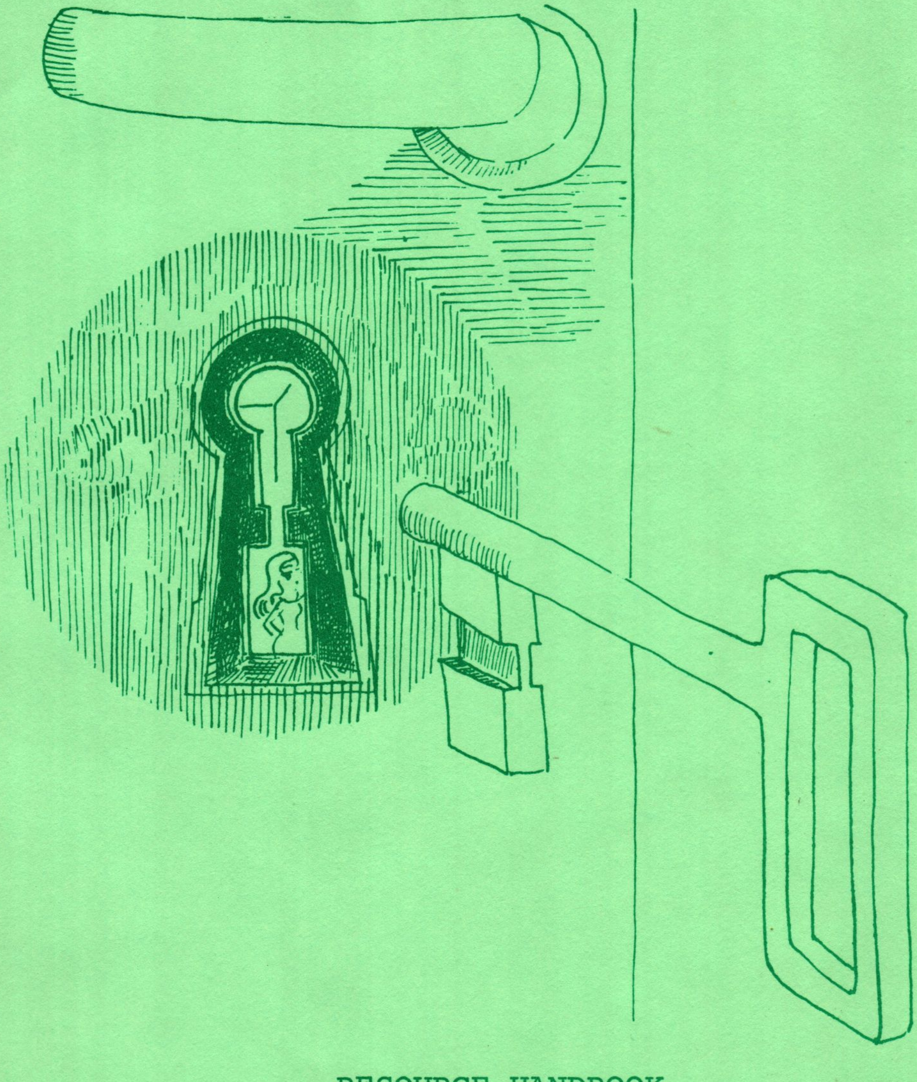
Additional publications may be obtained from:

Software Distribution Center
Digital Equipment Corporation
Maynard, Massachusetts 01754

Population: Self teaching BASIC Workbook	$2.00
BASIC Matrix Operations, Project Solo	$1.00
Problems for Computer Mathematics	$1.25
Advanced Problems for Computer Mathematics	2.00
BASIC Application Programs	
Mathematics I	$1.00
Mathematics II	1.00
Science	1.00
Business and Social Studies	1.00
Plotting	1.00
Huntington I Application Programs	
MATHEMATICS	$2.00
TEACHER ASSISTANCE	1.00
Huntington I Simulation Programs	
BIOLOGY	$1.00
CHEMISTRY	2.00
EARTH SCIENCE	1.00
PHYSICS	2.00
SOCIAL STUDIES	1.00
Huntington II Simulation Modules	
Student Workbook	$0.30
Teacher's Guide	.30
Resource Handbook	.50
Program Paper Tape	1.00

LOCKEY

THE LOCK AND KEY MODEL OF ENZYME ACTION



RESOURCE HANDBOOK

HUNTINGTON TWO COMPUTER PROJECT

Copyright C 1971, State University of New York

15 October 1971

The work of the Huntington Two Computer Project is partially supported by the National Science Foundation, Grant GW-5883.

Exclusive publishing rights granted to Digital Equipment Corporation.
REPRODUCTION NOT PERMITTED

RESOURCE MATERIALS

LOCKEY

TABLE OF CONTENTS

I. Background Information 1

- A. About the Enzyme 1
- B. About Lock and Key 2
- C. About Competitive Inhibition 3
- D. About the Millimole 3

II. Operation of *LOCKEY* 4

III. Sample Runs 6

IV. The *LOCKEY* Model 10

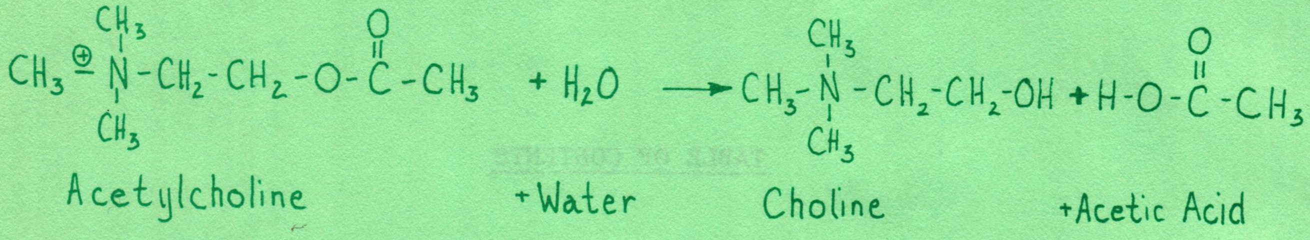
V. Program Listing 12

VI. Student Projects Using *LOCKEY* 15

VII. Bibliography .19

I. BACKGROUND INFORMATION

A. About the enzyme. Acetylcholinesterase (3,1,1,7-acetylcholine acetylhydrolase) catalyses the following reaction:



$$\mathrm{CH_3 \overset{\oplus}{-} \underset{CH_3}{\overset{CH_3}{N}} - CH_2 - CH_2 - O - \overset{O}{\overset{\|}{C}} - CH_3 + H_2O \longrightarrow CH_3 - \underset{CH_3}{\overset{CH_3}{N}} - CH_2 - CH_2 - OH + H - O - \overset{O}{\overset{\|}{C}} - CH_3}$$

Acetylcholinesterase is a hydrolytic enzyme as it uses water to break acetylcholine, and is therefore in the same catagory as most of the digestive enzymes. This was one of the primary reasons for the selection of the enzyme for this study. Results of this study might be applied with due restraint as a model for other hydrolytic digestive enzymes.

Acetylcholinesterase is thought to function primarily in the nervous system. Together with acetylcholine, it plays an important part in the transmission of impulses across the synapse, between neurons.

Impulse transmission along the nervous system is both electrical and chemical. Within the single neuron (nerve cell) the impulse is primarily electrical, but when the impulse reaches the end of the neuron it must cross a gap, called the synapse, in order to stimulate the next neuron and thus continue the transmission of the impulse. The first neuron cannot use its electrical impulse as it is too weak to cross even this small gap. The neuron must use a chemical system.

It is thought that the first neuron secretes the chemical substance acetylcholine. This chemical can quickly diffuse across the synapse, as the gap is small. When the acetylcholine reaches the second neuron on the other side of the synapse, the second neuron is stimulated and starts its own electrical impulse along the length of its cell.

The enzyme acetylcholinesterase must be present in the synapse or the acetylcholine secreted by the first neuron will continue to stimulate the second neuron and false electrical impulses will be created. In fact, physiologists have calculated that the acetylcholinesterase must complete the breakdown of the acetylcholine within 50 microseconds (.00005 seconds) to prevent these false impulses.

Should an inhibitor slow down the action of acetylcholinesterase destroying the acetylcholine in the synapse beyond this 50 microseconds, the controlled functioning of the nervous system would be impossible and death would be the ultimate outcome. (This simulation uses amounts of acetylcholine greater than that found in the synapse, but keep in mind that any slowing of the acetylcholinesterase function is a threat to life.)

Since high concentrations of acetylcholinesterase are found in nervous tissue, the enzyme is usually isolated from the electric eel. The enzyme has been isolated in a relatively pure state, but as yet no reliable determination has been made of the enzyme's size or number of catalytic sites. Luckily these figures are not of critical importance for this study as long as enzyme concentration is held constant during the set of experiments. Best estimates (as of 1969) hold the weight of the enzyme to be between 230,000 and 1,000,000 amu, depending on the method of isolation.

Much work has been done on acetylcholinesterase. This is due to the importance of the enzyme in nervous systems and also because it exhibits a phenomenon known as substrate inhibition. This means that at high acetylcholine concentrations the enzyme becomes less effective. This effect has been screened from this program by limiting the substrate concentration to a maximum of 3 millimolar concentration. When concentrations stay below this level, the enzyme is known to obey classical (Michaelis-Menten) kinetics. For this reason it is safe to use this program's behavior as a general, rather than a specific, case.

B. About Lock and Key. The lock and key hypothesis was one of the first attempts to explain enzyme specificity. First expounded by Emil Fisher in 1902, this model has served as the basis for most of the recent explanations of enzyme action.

It was noticed quite early in enzyme investigations that a specific enzyme worked on a small range of similar compounds, and usually only worked on one best. The lock and key model attempted to explain this by picturing the enzyme as a key and the proper substrate as a lock. Only the substrate (or those substances sufficiently like the substrate) could fit onto the enzyme and thus be catalysed. This is pictured as below:

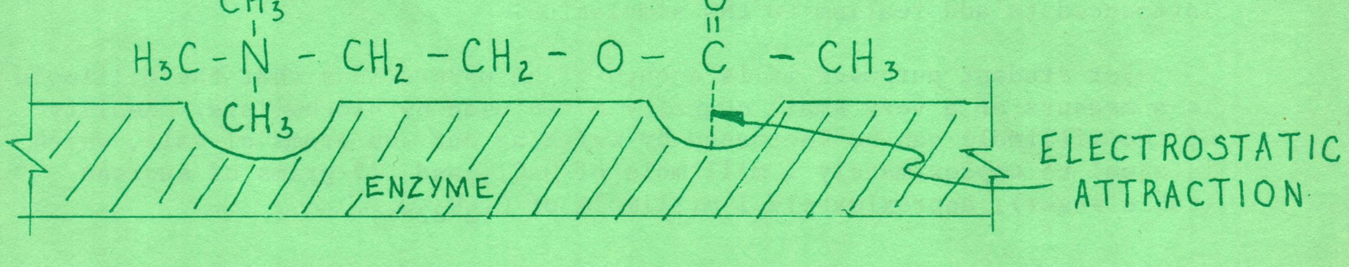


Diagram 3.1. The Lock and Key Model.

Today this rigid picture of the enzyme's catalytic site is not totally accepted and modifications have been made to allow the site to bend around the substrate. But the lock and key model of the catalytic site is good enough to permit understanding of many enzyme actions and is included in every potential biologist's background as his basis of understanding of the enzyme.

This program simulates an investigation to determine something of the nature of the enzyme's active site (the location at which catalysis occurs).

C. About Competitive Inhibition. Certain chemicals can alter the behavior of the enzyme. These chemicals that make the enzyme less effective are classified as inhibitors. In this experiment we are concerned with the class of inhibitors known as the competitive inhibitors.

These chemical compounds compete with the natural substrate for the active site on the enzyme. The more the inhibitor resembles natural substrate (in this case, acetylcholine), the better it will fit onto the enzyme, and the better the job it will do in inhibiting the interaction of the substrate and the enzyme.

In this program the student is given a set of inhibitors. By studying the effect of each inhibitor on the enzyme it is possible for certain deductions to be made about the active site on the enzyme.

D. About the Millimole. The millimole is literally 1/1000th of a mole (or 6.02×10^{20} molecules). The biochemist often makes use of this measure, rather than the more conventional mole, since in many instances it allows him to use whole numbers.

For the above reason, the millimole was selected as the standard of measure in LOCKEY. We are well aware that the concept of the mole is beyond students that are without high school chemistry, but the term was introduced to add realism to the simulation.

For student purposes it is enough if he understands that the millimole is a measure of a very small quantity often used by biochemists. Reality of the millimole can be increased by weighing out .06 grams of salt, which is equal to approximately 1 millimole of NaCl, and .25 grams of sucrose (table sugar), approximately 1 millimole of $C_{12}H_{22}O_{11}$.

II. OPERATION OF LOCKEY

Below are listed the meanings of the various inputs the student will be required to make.

1. "AMOUNT OF ACETYLCHOLINE (FROM 0 TO 3 MILLIMOLES)"

Acetylcholinesterase catalyses the following reaction:

acetylcholine + water → acetic acid + choline

Acetylocholine is the natural substrate that must be present for the enzyme to work. The student can choose any number between 1 and 3. (It is sufficient for the student to understand that a millimole is a very small measure of amount, often used by biochemists in enzyme studies.)

Any number from 1 to 3 millimoles will give meaningful results, as long as it is held constant throughout all of the student's experimental runs.

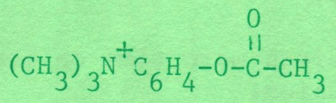
2. "THE TYPE OF INHIBITOR (USE CODE FROM 0 TO 5)"

The student has the choice of using any of five inhibitors listed below OR of not using any inhibitor by inputting zero. (Structural formulas can be found in the Student Material.)

1 - ammonium NH_4^+

2 - dimethylamine $(CH_3)_2NH_2^+$

3 - methylamine $CH_3NH_3^+$

4 - prostigmine 
$(CH_3)_3N^+C_6H_4\text{-}O\text{-}\overset{\overset{O}{\|}}{C}\text{-}CH_3$

5 - trimethylamine $(CH_3)_3NH^+$

The use of no inhibitor is important in the production of a control.

Note: In a real test tube experiment, these inhibitors would be added as chloride salts.

3. "AMOUNT OF INHIBITOR (IN MILLIMOLES)"

This will not appear if no inhibitor is selected.

Any positive number may be slected. The most appropriate number will vary according to:

a) the amount of acetylcholine selected,

b) the experimental approach selected by the student

In general it is a good idea to select an amount of inhibitor that is at least 100 times greater than the amount of substrate.

Output Format - The student can select either a table or a computer graph. Both formats will produce suitable data which will enable him to compile his results on a single graph. Amount of product (acetic acid) is placed on the vertical axis and time on the horizontal axis.

The student can take two possible experimental approaches. (It might be useful to have your class divide into two groups, one for each approach.)

1. The student can select a certain amount of inhibitor and hold this constant throughout the investigation. He must then graph his results as product versus time and compare the results of each of the inhibitors versus the control in which no inhibitor was used.

2. Vary the amounts of each inhibitor until he slows the enzyme to one half its normal level (without inhibitor). This is very close to the I_{50} level that is commonly determined by biochemists when they want to evaluate the effectiveness of an inhibitor substance. I_{50} stands for the concentration of chemical necessary to inhibit 50%. If the student takes this approach all he need do is match the I_{50} values for the five inhibitors in order to determine the most effective. (The compound with the lowest I_{50} is the most effective.)

Approach 1 uses less time on the machine and more time in graphing and reaching conclusions.

Approach 2 requires more time on machine but is much easier in terms of reaching a conclusion.

III. SAMPLE RUNS

These runs simulate a typical student's experiment. The results of these runs have been compiled in Graph 2.1, p.4, Section II.

At higher inhibitor concentrations (for the same amount of acetylcholine), the differences between inhibitors would be even more noticeable.

```
LOCKEY- A COMPETITIVE INHIBITION STUDY OF THE ENZYME
ACETYLCHOLINESTERASE FEATURING THE LOCK AND KEY HYPOTHESIS

DO YOU WISH INSTRUCTIONS? (1=YES, 0=NO)? 0

*******************************************************

AMOUNT OF ACETYLCHOLINE - FROM 0 TO 3 MILLIMOLES? 2
TYPE OF INHIBITOR - USE CODE FROM 0 TO 5? 0
DATA FORMAT: 1=TABLE, 2=GRAPH   TYPE NUMBER OF CHOICE? 1

MINUTES       ACETYLCHOLINE    TOTAL ACETIC
ELAPSED       REMAINING        ACID PRODUCED
-------       -------------    -------------
 0              2               0
 .1             1.72            .28
 .2             1.43            .57
 .3             1.15            .85
 .4             .88             1.12
 .5             .61             1.39
 .6             .36             1.64
 .7             .14             1.86
 .8             .02             1.98
 .9             0               2
 THE REACTION HAS RUN TO COMPLETION

CONCENTRATION OF INHIBITIOR REMAINING: 0 MILLIMOLES

ANOTHER EXPERIMENT? (1=YES, 0=NO)? 1
```

```
********************************************************

AMOUNT OF ACETYLCHOLINE - FROM 0 TO 3 MILLIMOLES? 2
TYPE OF INHIBITOR - USE CODE FROM 0 TO 5? 1
AMOUNT OF INHIBITOR IN MILLIMOLES? 100
DATA FORMAT: 1=TABLE, 2=GRAPH? 1


MINUTES     ACETYLCHOLINE   TOTAL ACETIC
ELAPSED     REMAINING        ACID PRODUCED
-------     -------------   -------------
 0              2             0
 .1             1.72          .28
 .2             1.44          .56
 .3             1.16          .84
 .4             .88           1.12
 .5             .62           1.38
 .6             .37           1.63
 .7             .16           1.84
 .8             .03           1.97
 .9             0             2
 THE REACTION HAS RUN TO COMPLETION

CONCENTRATION OF INHIBITIOR REMAINING: 100 MILLIMOLES

ANOTHER EXPERIMENT? (1=YES, 0=NO)? 1

********************************************************

AMOUNT OF ACETYLCHOLINE - FROM 0 TO 3 MILLIMOLES? 2
TYPE OF INHIBITOR - USE CODE FROM 0 TO 5? 2
AMOUNT OF INHIBITOR IN MILLIMOLES? 100
DATA FORMAT: 1=TABLE, 2=GRAPH? 2

MIN.        MILLIMOLES ACETIC ACID PRODUCED
      0                        1                        2
      I....I....I....I....I....I....I....I....I....I....I
 0   I* 0
 .1  I      * .23
 .2  I            * .46
 .3  I                 * .68
 .4  I                      * .89
 .5  I                           * 1.09
 .6  I                                * 1.27
 .7  I                                    * 1.44
 .8  I                                        * 1.58
 .9  I                                           * 1.71
 1   I                                             * 1.8
 1.1 I                                               * 1.87
 1.2 I                                                * 1.92
 1.3 I                                                 * 1.95
 1.4 I                                                 * 1.97
 1.5 I                                                  * 1.98
 1.6 I                                                  * 1.99
 1.7 I                                                  * 2
 THE REACTION HAS RUN TO COMPLETION

CONCENTRATION OF INHIBITIOR REMAINING: 100 MILLIMOLES

ANOTHER EXPERIMENT? (1=YES, 0=NO)? 1
```

```
*******************************************************

AMOUNT OF ACETYLCHOLINE - FROM 0 TO 3 MILLIMOLES? 2
TYPE OF INHIBITOR - USE CODE FROM 0 TO 5? 3
AMOUNT OF INHIBITOR IN MILLIMOLES? 100
DATA FORMAT: 1=TABLE, 2=GRAPH? 2

MIN.        MILLIMOLES ACETIC ACID PRODUCED
      0                        1                        2
      I....I....I....I....I....I....I....I....I....I....I
 0   I* 0
 .1   I       * .27
 .2   I              * .54
 .3   I                    * .81
 .4   I                           * 1.07
 .5   I                                 * 1.32
 .6   I                                       * 1.55
 .7   I                                            * 1.75
 .8   I                                                * 1.9
 .9   I                                                 * 1.98
 1    I                                                  * 2
 THE REACTION HAS RUN TO COMPLETION

CONCENTRATION OF INHIBITIOR REMAINING: 100 MILLIMOLES

ANOTHER EXPERIMENT? (1=YES, 0=NO)? 1

*******************************************************

AMOUNT OF ACETYLCHOLINE - FROM 0 TO 3 MILLIMOLES? 2
TYPE OF INHIBITOR - USE CODE FROM 0 TO 5? 4
AMOUNT OF INHIBITOR IN MILLIMOLES? 100
DATA FORMAT: 1=TABLE, 2=GRAPH? 2

MIN.        MILLIMOLES ACETIC ACID PRODUCED
      0                        1                        2
      I....I....I....I....I....I....I....I....I....I....I
 0   I* 0
 .1   I* 0
 .2   I* 0
 .3   I* 0
 .4   I* 0
 .5   I* .01
 .6   I* .01
 .7   I* .01
 .8   I* .01
 .9   I* .01
 1    I* .01
 1.1 I* .01
 1.2 I* .01
 1.3 I* .01
 1.4 I* .01
 1.5 I* .02
 1.6 I* .02
 1.7 I* .02
 1.8 I* .02
 1.9 I * .02
 2    I * .02
DO YOU WANT TO CONTINUE THE ASSAY? (1=YES, 0=NO)? 0

CONCENTRATION OF INHIBITIOR REMAINING: 100 MILLIMOLES
```

```
********************************************************

AMOUNT OF ACETYLCHOLINE - FROM 0 TO 3 MILLIMOLES? 2
TYPE OF INHIBITOR - USE CODE FROM 0 TO 5? 5
AMOUNT OF INHIBITOR IN MILLIMOLES? 100
DATA FORMAT: 1=TABLE, 2=GRAPH? 2

MIN.        MILLIMOLES ACETIC ACID PRODUCED
      0                        1                        2
      I....I....I....I....I....I....I....I....I....I....I
 0   I* 0
 .1  I   * .11
 .2  I     * .21
 .3  I        * .31
 .4  I          * .4
 .5  I            * .49
 .6  I               * .58
 .7  I                 * .66
 .8  I                   * .74
 .9  I                     * .82
 1   I                      * .89
 1.1 I                        * .96
 1.2 I                          * 1.03
 1.3 I                           * 1.09
 1.4 I                             * 1.15
 1.5 I                              * 1.21
 1.6 I                                * 1.26
 1.7 I                                 * 1.31
 1.8 I                                  * 1.36
 1.9 I                                   * 1.4
 2   I                                    * 1.45
DO YOU WANT TO CONTINUE THE ASSAY? (1=YES, 0=NO)? 1
 2.1 I                                     * 1.48
 2.2 I                                      * 1.52
 2.3 I                                       * 1.56
 2.4 I                                        * 1.59
 2.5 I                                        * 1.62
 2.6 I                                         * 1.65
 2.7 I                                          * 1.67
 2.8 I                                          * 1.7
 2.9 I                                           * 1.72
 3   I                                            * 1.74
DO YOU WANT TO CONTINUE THE ASSAY? (1=YES, 0=NO)? 0

CONCENTRATION OF INHIBITIOR REMAINING: 100 MILLIMOLES

ANOTHER EXPERIMENT? (1=YES, 0=NO)? 0
```

IV. THE LOCKEY MODEL

The program LOCKEY is based both on a fundamental theory of enzyme action (the Michaelis-Menten equation) and a set of actual data from the hydrolytic enzyme acetylcholinesterase.

The choice of acetylcholinesterase was fortunate for several reasons:

1) Being hydrolytic, it is similar to the digestive enzymes, but its behavior is far simpler.

2) A great amount of study has been made of this enzyme, so most of the measurements needed were available.

3) Acetylcholinesterase obeys the above theory over the ranges of substrate concentrations used in the program.

LOCKEY assumes the following interactions are taking place within the experimental test tube:

$$E + S \rightleftharpoons [CC] \longrightarrow E + P \qquad (1)$$

$$E + I \rightleftharpoons EI \qquad (2)$$

where E=enzyme
S=substrate (acetylcholine)
[CC]=catalytic complex (enzyme + acetylcholine undergoing catalysis)
P=products (acetic acid and choline)
I=inhibitor chemical
EI=the combination of enzyme and inhibitor.

The essential meaning of these two equations, if you feel it necessary for your students to know them, is that the enzyme can combine with the natural substrate (as in Eqn. 1) or with the inhibitor chemical (as in Eqn. 2) but not with both. So it is said that the substrate and the inhibitor are in competition for the enzyme.

If the inhibitor is a good competitor, only small amounts of substrate will be able to react with the enzyme, so small amounts of product will be produced. Or in other words, the better the inhibitor the less product that will be produced in a given time.

Using the lock and key theory we assume that the best inhibitor will be the one that has the closest resemblance to the natural substrate.

Assumptions

1. Products of the reaction are immediately used so that there is no measurable back reaction (this assumption is always necessary for the use of Michaelis-Menten enzyme kinetics).

2. Steady state concentrations are maintained throughout the reaction (another necessary assumption).

3. pH is held constant at 7.0 and temperature at 25°C. (Traditional laboratory conditions for the study of this enzyme.)

4. Enzyme concentration is approximately $10^{-8}\underline{M}$ and total amount of enzyme is a constant. (The approximate value is necessary because the actual weight of the enzyme has not yet been reliably determined.)

Variable Designation		Program Symbol
V	Moles product produced/minute.	V1
V_{max}	Maximal rate of product production at a given enzyme concentration & infinite substrate concentration.	M
$[S]$	Concentration of substrate (molar).	S1
K_m	Michaelis Constant; varies according to nature of substrate and enzyme being used.	K1
$[I]$	Molar concentration of inhibitor.	I
K_I	Binding constant of inhibitor to enzyme. Actually equal to $K_{diss} = \frac{[E]\ [I]}{[EI]}$ Varies with the type of inhibitor used.	K2

Equations Used

$$V = \frac{V_{max}\ [S]}{[S] + K_m \left[1+\frac{[I]}{K_1}\right]}$$

$$V1 = \frac{(M*S1)}{(S1+K)}$$

$$K = K1\left[1+\frac{I}{K2}\right]$$

V. PROGRAM LISTING

Special Language Feature: RESTORE

```
100 REM -LOCKEY - STUDY OF COMPETITIVE INHIBITION OF THE ENZYME
110 REM ACETYLCHOLINESTERASE FOR APPLICATION TO LOCK AND KEY MODEL
120 REM COPYRIGHT 1971 - STATE UNIVERSITY OF NEW YORK
130 REM PROGRAM DEVELOPED BY J. FRIEDLAND AND B. ROSEN
140 REM PROGRAMMED BY D. SOBIN AND J. FRIEDLAND 8/71
150 REM LATEST REVISION 8-25-72
160 PRINT "LOCKEY- A COMPETITIVE INHIBITION STUDY OF THE ENZYME"
170 PRINT "ACETYLCHOLINESTERASE FEATURING THE LOCK AND KEY HYPOTHESIS"
180 PRINT
190 PRINT "DO YOU WISH INSTRUCTIONS? (1=YES, 0=NO)";
200 INPUT Q
210 IF Q=0 THEN 610
220 IF Q<>1 THEN 190
230 PRINT
240 PRINT "  YOU ARE CONDUCTING AN INVESTIGATION OF THE ENZYME"
250 PRINT " ACETYLCHOLINESTERASE.  FROM THE NAME YOU CAN TELL"
260 PRINT " THAT THIS ENZYME WORKS ON THE CHEMICAL ACETYLCHOLINE."
270 PRINT " IT BREAKS ACETYLCHOLINE INTO ACETIC ACID AND CHOLINE."
280 PRINT
290 PRINT "  WE WANT TO INVESTIGATE WHICH INHIBITOR IS THE MOST"
300 PRINT " EFFECTIVE IN SLOWING THE NORMAL ACTION OF THE ENZYME."
310 PRINT " THIS WILL GIVE US VALUABLE INFORMATION ON ITS ACTION."
320 PRINT
330 PRINT "       THE CODE FOR THE INHIBITORS IS:"
340 PRINT
350 PRINT "          1= AMMONIUM"
360 PRINT "          2= DIMETHYLAMINE       BE SURE TO CONSULT"
370 PRINT "          3= METHYLAMINE         THE IMPORTANT STRUCTURAL"
380 PRINT "          4= PROSTIGMINE         FORMULAE LISTED IN THE"
390 PRINT "          5= TRIMETHYLAMINE      INFORMATION PACKET"
400 PRINT "          0= NO INHIBITOR"
410 PRINT
420 PRINT " IN THIS STUDY YOU CAN CONTROL:"
430 PRINT "        THE AMOUNT OF ACETYLCHOLINE"
440 PRINT "        THE TYPE OF INHIBITOR"
450 PRINT "        AND THE AMOUNT OF INHIBITOR"
460 PRINT
470 PRINT "BY COMPARING THE STRUCTURE OF ACETYLCHOLINE WITH"
480 PRINT "THE STRUCTURE OF THE FIVE INHIBITORS YOU SHOULD"
490 PRINT "BE ABLE TO MAKE A HYPOTHESIS AS TO WHICH OF THE "
500 PRINT "FIVE INHIBITORS WILL BE THE MOST EFFECTIVE"
510 PRINT
520 PRINT "   REMEMBER: INCLUDE YOUR KNOWLEDGE OF THE"
530 PRINT "   LOCK AND KEY MODEL OF ENZYME ACTION."
540 REM S1=AMOUNT OF SUBSTRATE
550 REM P1=AMOUNT OF PRODUCT AT TIME T1
560 REM V=VELOCITY OF REACTION IN MILLIMOLES/.002 MIN
570 REM M=MAX VELOCITY AT GIVEN ENZYME CONCENTRATION
580 REM K1=MECHAELIS-MENTEN CONSTANT
590 REM K2=BINDING CONSTANT OF INHIBITIOR
600 REM I=CONCENTRATION OF INHIBITOR
610 LET N=1
```

```
620 PRINT
630 READ T,P1,C,K1,M,X,V
640 DATA 0,0,0,9E-5,3E-3,1000,0
650 PRINT "*********************************************************"
660 LET T1=0
670 PRINT
680 PRINT "AMOUNT OF ACETYLCHOLINE - FROM 0 TO 3 MILLIMOLES";
690 INPUT S1
700 IF S1>3 THEN 680
710 IF S1<0 THEN 680
720 IF S1>0 THEN 750
730 PRINT "NO REACTION IS POSSIBLE WITHOUT SUBSTRATE"
740 GOTO 1470
750 LET S1=S1/1000
760 LET S2=S1
770 PRINT "TYPE OF INHIBITOR - USE CODE FROM 0 TO 5";
780 INPUT K2
790 IF K2<>INT(K2) THEN 770
800 IF K2>5 THEN 770
810 IF K2<0 THEN 770
820 IF K2=0 THEN 1060
830 IF K2<4 THEN910
840 IF K2=4 THEN 880
850 REM CODE 5= TRIMETHYLAMINE
860 LET K2=2.6E-3
870 GOTO 1010
880 REM CODE 4= PROSTIGMINE
890 LET K2=1.6E-5
900 GOTO 1010
910 IF K2>=2 THEN 950
920 REM CODE 1 = NH3
930 LET K2=.9
940 GOTO 1010
950 IF K2=2 THEN 990
960 REM CODE 3= METHYLAMINE
970 LET K2=.12
980 GOTO 1010
990 REM CODE 2= DIMETHYLAMINE
1000 LET K2=2.1E-2
1010 PRINT "AMOUNT OF INHIBITOR IN MILLIMOLES";
1020 INPUT I
1030 LET I=I/1000
1040 IF I<0 THEN 1010
1050 IF I<>0 THEN 1080
1060 LET K2=1
1070 LET I=0
1080 LET K=K1+(K1*I/K2)
1090 PRINT "DATA FORMAT: 1=TABLE, 2=GRAPH";
1100 IF N<>1 THEN 1120
1110 PRINT "   TYPE NUMBER OF CHOICE";
1120 INPUT Q
1130 IF (Q-1)*(Q-2)<>0 THEN 1090
1140 IF Q=1 THEN 1290
1150 GOTO 1630
1160 IF Q=2 THEN 1670
1170 GOTO 1340
```

```
1180 IF T1=X THEN 1390
1190 FOR T=T1+1 TO T1+50
1200 REM FOLLOWING EQN EQUIV. TO V=M*S/S+K1(1+I/K2) = M-M EQN
1210 LET V1=(M*S1)/(S1+K)
1220 LET V=(V+V1)/1000
1230 LET S1=S1-V
1240 LET P1=P1+V
1250 LET V=V1
1260 NEXT T
1270 LET T1=T1+50
1280 GOTO 1160
1290 PRINT
1300 PRINT
1310 PRINT"MINUTES    ACETYLCHOLINE   TOTAL ACETIC"
1320 PRINT"ELAPSED    REMAINING       ACID PRODUCED"
1330 PRINT"-------    -------------   -------------"
1340 PRINT (T1)/500, INT(S1*10↑5+.5)/100,INT(P1*10↑5+.5)/100
1350 IF INT(S1*10↑5+.5)/100<=0 THEN 1370
1360 GOTO 1180
1370 PRINT " THE REACTION HAS RUN TO COMPLETION"
1380 GOTO 1450
1390 PRINT "DO YOU WANT TO CONTINUE THE ASSAY? (1=YES, 0=NO)";
1400 INPUT Q2
1410 IF Q2=0 THEN 1450
1420 IF Q2<>1 THEN 1390
1430 LET X=X+500
1440 GOTO 1190
1450 PRINT
1460 PRINT "CONCENTRATION OF INHIBITIOR REMAINING:"I*1000;"MILLIMOLES"
1470 PRINT
1480 PRINT "ANOTHER EXPERIMENT? (1=YES, 0=NO)";
1490 INPUT Q
1500 IF Q=1 THEN 1600
1510 IF Q<>0 THEN 1460
1520 PRINT
1530 PRINT
1540 PRINT
1550 PRINT
1560 PRINT
1570 PRINT
1580 PRINT
1590 STOP
1600 RESTORE
1610 LET N=N+1
1620 GOTO 620
1630 PRINT
1640 PRINT"MIN.         MILLIMOLES ACETIC ACID PRODUCED"
1650 PRINT TAB(6);"0";TAB(30);INT(S2*10↑3+.99)/2;TAB(55);
1655 PRINTINT(S2*10↑3+.99)
1660 PRINT "    I....I....I....I....I....I....I....I....I....I....I"
1670 PRINT (T1)/500;TAB(5);"I";TAB(P1*10↑3/INT(S2*10↑3+.99)*50+6.5);
1675 PRINT"*";
1680 PRINT INT(P1*10↑5+.5)/100
1690 GOTO 1350
1700 END
```

VI. STUDENT PROJECTS USING LOCKEY

1. Determination of the I_{50}.

The I_{50} is used by biochemists as a first quantitative approximation to the strength of an inhibitor. As such, it is rather easy to obtain and is suitable for a first student independent project, after completion of the program LOCKEY in class.

The class treatment of LOCKEY was essentially a qualitative determination of relative inhibitor strength. From this experiment the student determined the order of strength of the various inhibitors he tested. But the question might arise: How does inhibitor #5 in our investigation compare to compound X, which we did not test? With only qualitative determinations we may have to repeat all or some of our previous experiments each time such a question arises.

The use of the I_{50} can overcome this objection. I_{50} is literally the amount of inhibitor that causes 50% inhibition of the enzyme activity. This measure can be found for all the inhibitors. Clearly the best inhibitor will be the one with the lowest measured I_{50}.

Object - Experimentally determine the I_{50} for each of the inhibitors.

Suggested Technique - The following is not the only proper approach, but it has been trial-tested with LOCKEY with good success. Creative students should be encouraged to try other techniques with the proviso that good written notes be taken.

a. Normal activity determination - Normal activity under experimental conditions will be determined from a control run with no inhibitor used. Any amount of acetylcholine can be used, but once selected must be held constant throughout the rest of the investigation. Determination of activity can be made either from graph or table data.

Enzyme activity can be defined in many ways; we will use the following:

i. Activity = millimoles of product produced per minute = P/min. This can be figured by either reading the value for the graph or table or by using simple math. Just reading the product value at 1 minute has a real disadvantage. As you may have noticed, the reaction slows down as it progresses. We can eliminate this slowing effect due to low levels of acetylcholine by using the following simple equation to figure activity:

ii. Activity = amount of product in first 1/10th minute x 10.

b. Figuring the value of the I_{50} - From (ii) above we should know the normal activity of the enzyme under these experimental conditions. We want to determine the amount of each inhibitor that will cut the activity to 50% of its normal value:

iii. activity with inhibitor present = normal activity/2.

c. Experiments with the inhibitors - Investigate one inhibitor at a time to find out the amount (in millimoles) of inhibitor that is necessary to reduce enzyme activity to one half its normal value.

Remember to use the same amount of acetylcholine as you used in the control and to figure the activity after each experiment in the same fashion as you did with the control.

(Note: This project may take large amounts of computer time, and therefore is usually unsuitable for a laboratory for a whole class. Time may be cut by investigating one or a few inhibitors instead of all five. But even with this revision, it is felt that this is only suitable for an individual or small group project.)

2. Advanced activity determination of I_{50}.

In the previous experiment, we used a simple formula to determine activity. In this experiment we examine only the first .1 minute and then multiply the result to find the product for one minute. In reality scientists measure activities using much shorter time periods than .1 minute, because even at .1 minute the reaction has often slowed due to the lowering of the acetylcholine level.

To get more accurate results there are two possible approaches:

a. More accurate equipment for shorter time measurements. This can be simulated by making the changes indicated in the following lines of the program:

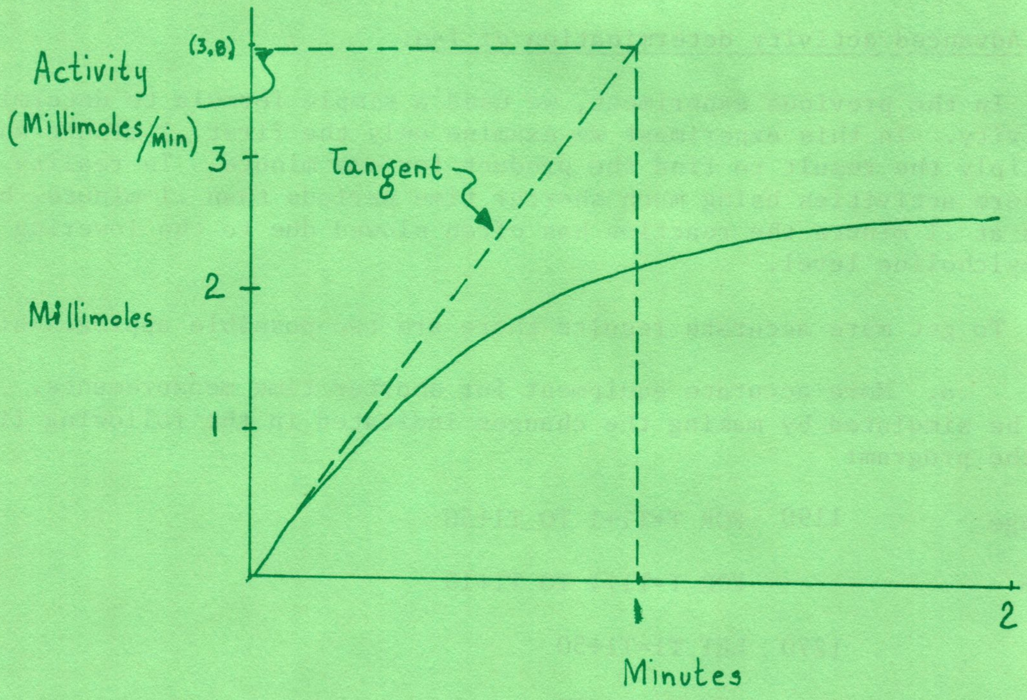
```
Change        1190  FOR T=T1+1 TO T1+50

  to                FOR T=T1+1 TO T1+10

              1270  LET T1=T1+50

  to                LET T1=T1+10

              1430  LET X=X+500

  to                LET X=X+100
```

In line 640 change the number 1000 to 100.

This approach is the more expensive of the two, so it is not often the method of choice.

b. Graphical method - Drawing an estimated tangent to the curve, as near as possible to the origin, is often found to be a satisfactory technique. To get this technique to work, obtain data as a table and plot

b. Graphical method - drawing an estimated tangent to the curve, as near as possible to the origin, is often found to be a satisfactory technique. To get this technique to work, obtain data as a table and plot the data yourself (the computer plot is not accurate enough). Draw your tangents as below. By extending the tangent to 1 minute on the graph, you can read the activity directly:



Graph 3.1. The graphical method.

3. Investigation of how activity varies with initial acetylcholine concentration.

Since this investigation is not concerned with inhibition or with the inhibitors, all experiments will use CODE 0 (ZERO) on the question TYPE OF INHIBITOR:

We will vary the amount of substrate in each of the experiments. All other factors will be kept constant.

Procedure:

1. Output data of each experiment in table format.
2. Graph each experiment separately on fine line graph paper, as amount of product vs. time (see previous experiment).
3. Figure the activity graphically as in the previous experiment.
4. Compose a data table in the following format from all of the activity determinations (Step 3):

Millimoles Acetylcholine	Activity Per Minutes (millimoles/min.)

5. Plot the data with activity on the vertical axis and substrate on the horizontal axis.

(It may be necessary to make the indicated changes in lines 1190, 1270, 1430, and 640 of the program. [See (a) of project 2 above.])

4. The Lineweaver-Burke plot of activity versus substrate.

Follow the same general approach as above, but plot 1/activity versus 1/acetylcholine concentration. The graph should be a straight line.

These are only a few of the possible variations of LOCKEY for individual project work. A glance at the enzyme kinetics section in any introductory biochemistry text will suggest still other experiments. Generally if the experiment involves activity, inhibitors or substrate concentrations, it may be modeled using LOCKEY with minimum modification of inputs or data formats.

VII. BIBLIOGRAPHY

A. Secondary School Textbooks

Biological Science: An Inquiry into Life (BSCS Yellow) 2nd edition, New York: Harcourt, Brace and World, 1968, pp. 105-108

A very good discussion of an appropriate enzyme-substrate model. See diagram, pp. 108 and 109.

Biological Science: Molecules to Man (BSCS Blue) Revised edition, Boston: Houghton Mifflin Company, 1968, pp. 145-146.

Discussion similar to that above. Above pages include several useful diagrams explaining the lock and key model of enzyme action.

Concepts in Modern Biology, Kraus, David and Perkins, Otho. Cambridge Book Company, Inc., 1969, pp. 39-43.

Comprehensive summary of several aspects of enzyme action, including the lock and key model.

Modern Biology, Otto, J. and Towle, A., New York: Holt, Rinehart and Winston, Inc., 1969, Chapter 3.

B. College Level Texts

Biological Science, Keeton, William T., New York: W. W. Norton and Company, 1967, pp. 45-50.

A general discussion of how chemical reactions relate to the study of biology. The theory of a catalyst is related to enzyme models. Text includes many interesting facets that may "open up" some of the optional exercises suggested for LOCKEY.

The Science of Biology, Weisz, Paul B. New York: McGraw-Hill Book Company, 1967, pp. 103-108.

A more detailed analysis of enzyme action in biological systems. Different types of inhibition are discussed.

The Cell, Swanson, C., Englewood Cliffs, N. J.: Prentice-Hall, 1968.

Physiology of Man, 3rd edition. Langley, L. L. and Cheraskin, E., New York: Reinhold Publishing Corp., 1965, pp. 68-78.

A general textbook of human physiology. Highly useful as a teacher reference book for many aspects of secondary school biology.

C. Biochemistry Texts. All of the below will supply specific information on many aspects of enzyme action and competitive inhibition. Please consult the index of each of these texts to find appropriate materials.

Biological Chemistry, Mahler, H. And Cordes, E., New York: Harper and Row, Publishers, 1966.

Principles of Biochemistry, White, A., Handler, P. and Smith, E., 3rd edition. New York: McGraw-Hill Book Company, 1964.

Textbook of Biochemistry, Harrow, B. and Mazur, A., 9th edition. Philadelphia: W. B. Saunders Company, 1966.

D. Periodicals

"Nerve Cells and Behavior," Kandel, Eric R. Scientific American, July 1970, Vol. 223, No.1, p.57.

"The Synapse," Eccles, John, Scientific American, January 1965, Vol. 212, No.1, p.56.